

Section B

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CARNITINE SUPPLEMENTATION IN SPORT

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Abstract

Carnitine is a compound that can be either endogenous, synthesized by the liver and kidneys, both exogenous, introduced with the diet, in particular through meat and dairy products. Carnitine has a dual role as it is required for long-chain fatty acid oxidation, and also shuttles accumulated acyl groups out of the mitochondria. Muscle requires optimization of both of these metabolic processes during peak exercise performance. Theoretically, carnitine availability may become limiting for either fatty acid oxidation or the removal of acyl-CoAs during exercise. Despite the theoretical basis that carnitine supplementation improves performance of exercise, clinical data have not shown significant benefits in this regard in healthy subjects, nor for strength performance, nor for endurance performance.

Keywords: Carnitine, supplementation, fatty acid oxidation, FABPc, β -HAD performance.

Introduction

Carnitine (L-3-hydroxytrimethylamminobutan oate) (Figure 1) is an endogenous compound with a specific role in the metabolism: it is derived from food sources as well as from endogenous biosynthesis. Important dietary sources of this compound, are meat and dairy products (Rebouche et al., 1984). Lysine supply the biosynthetic precursor of the carbon backbone of carnitine; the final step of its biosynthesis, occurs in the liver and kidney (Rebouche et al., 1986).

The loss of carnitine in humans occurs through urinary excretion of carnitine and acylcarnitine. Carnitine and acylcarnitine are both filtered and reabsorbed in the renal

tubule (Ohtani et al., 1984). Different tissues have different transport systems (Brass E et al 1992), which imply a difference in the content of carnitine in tissues, metabolic availability and turnover rates.

The differences in the various tissues are emphasized by a comparison between the values of total carnitine (carnitine and acylcarnitine): for example in the plasma is 60 mol/L, in the liver is 900 mmol/kg and in skeletal muscle is 4000 mmol/kg. The tissue of the myocardial and skeletal muscle represent the locations of greatest energy demand (Fraenkel and Friedman, 1957; Rebouche and Seim, 1998).

Carnitine can be synthesized in the kidney and liver thanks to the action of the enzyme Acetyl-L-Carnitine transferase (ALC transferase) from lysine, methionine, vitamin C and other substances that act as a substrate or cofactors.

The carnitine complex is in the L-carnitine, its esters (L-propionilcarnitine and L-acetylcarnitine) and by an articulated enzyme system, which is located at the level of the mitochondrial membrane, and includes: Carnitine palmitoyltransferase I and II (CPT I-II), Carnitine/acylcarnitine translocase (CT), carnitine acetyl transferase (CAT).

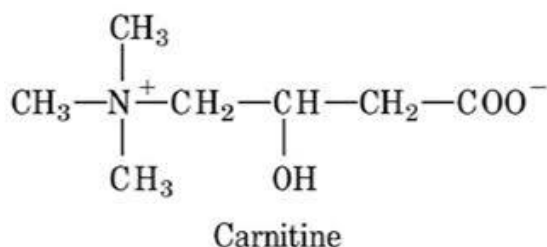


Figure 1 Carnitine structure

Why carnitine is used to improve the performance?

Various specific methods have been studied to explain the effects of carnitine on athletic performance. The role of carnitine on mitochondrial oxidation of fatty acids suggests that supplementation could increase the oxidation, thereby increasing the ATP available for the mechanical work (Gorostiaga et al., 1989).

The β -oxidation process consists in sequential shortening of the fatty acid chain with production of acetyl-CoA. Since the activated long chain fatty acids are not able to cross the inner mitochondrial membrane, the process of shortening of their chain is preceded by their carnitine-dependent transport that are activated in mitochondrial transmembrane space.

The process start with the formation of acyl-CoA by acyl-CoA synthetase (LCAS) that is localized in the external mitochondrial membrane. Subsequently is involved a

voltage-dependent anion channel through which the movement of fatty acids activated takes place through the external mitochondrial membrane. In this step, fatty acids are processed in acylcarnitine with CPT-I enzyme it also localized in the external mitochondrial membrane. The reaction products, carnitine esters, are transported into the mitochondrial matrix through an exchange reaction in which is involved carnitine-acylcarnitine translocase (CACT), a protein located in the inner membrane.

Inside the matrix, the esters of acylcarnitine are transformed in their respective CoA esters through CPT-II enzyme, an isoform of CPTI enzyme, that is associated with the inner side of mitochondrial membrane. The β -oxidation process of very long chain fatty acids occurs mainly in peroxisomes; while, the long-chain fatty acids are oxidized both in peroxisomes and in mitochondria.

However, unlike the mitochondrial β -oxidation, the oxidation of fatty acids that occurs in peroxisomes is incomplete, because it get acetyl-CoA and acyl-CoA short and medium chain. Therefore, to obtain the complete oxidation to CO_2 , the products of peroxisomes should be transported into the mitochondria where esters of CoA will be finally oxidized in CO_2 and H_2O through the β -oxidation, the Krebs cycle and respiratory chain.

If carnitine supplementation increase muscle oxidation of fatty acids, this could also delay the use of muscle glycogen and thus delay the onset of fatigue (Marconi C et al., 1985). Maintaining the rate of β -oxidation, carnitine allows the utilization of glucose and then preserves the muscle glycogen well as provides maximum production of ATP from metabolism of fats (Costill D et al., 1977).

Different approaches have been used to evaluate the effect on fatty acid oxidation by carnitine supplementation. During an exercise perform below the anaerobic threshold, the respiratory quotient reflect the relative rate of glucose oxidation (Wasserman K, 1975); also

the rate of oxygen consumption can be used to estimate the absolute velocity of oxidation of fatty acids (Livesey G et al., 1988). There are no experimental data which support that the assumption of carnitine increase fat oxidation or preserve muscle glycogen during exercises performance in healthy subjects. An alternative mechanism through which the carnitine could be taken to improve the physical performance, could be the production of short-chain acylcarnitine. The formation of acetylcarnitine dall'acetyl-CoA, may represent an alternative product of the oxidation.

The acetyl-CoA is the common product of glycolysis (from the oxidative decarboxylation of pyruvate) and β -oxidation of fatty acids: it is the substrate used for the next complete oxidation in the tricarboxylic acid cycle. If the production of acetyl-CoA exceeds the capability of use it in tricarboxylic acid cycle, there will be an increase of its concentration. This could inhibit the activity of pyruvate dehydrogenase, the enzyme that catalyzes the oxidation of pyruvate to acetyl-CoA (Bremer J, 1969).

When the activity of pyruvate dehydrogenase decrease, pyruvate should reduced to lactate by the process of fermentation. During an high intensity exercise, there is a correlation between lactate accumulation and acetyl-CoA production (Constantin-Teodosiu et al., 1991). The production of acetylcarnitine could potentially control the content of acetyl-CoA through the inhibition of pyruvate dehydrogenase and increasing the availability of coenzyme A. The importance of the production of acetylcarnitine from mitochondrial oxidation has been demonstrated using *in vitro* approach. However, the demonstration of these effects depends of various conditions, not only physiological, including high concentrations of the endogenous carnitine.

The esogenous high concentrations of carnitine, have no effect on maximum rates of

oxidation and also on the content of the mitochondrial acetyl-CoA in liver and also in isolated mitochondria (EP Brass et al 1980). However, other experiments that increased several times hepatic carnitine content, had not effects on acetyl-CoA content, probably because it rapidly reaches equilibrium (EP Brass et al., 1980). The flow of pyruvate from pyruvate dehydrogenase action during an exercise near maximal loads of works, has been estimated at 1.9 mmol/kg in a minute (Putman et al., 1995). The decrease of acetyl-CoA production, influence the rate of the oxidation of fatty acids and convert in only 2 minutes the content of muscle carnitine (about 4 mmol/kg) in acetylcarnitine.

Furthermore, the quantity of carnitine necessary to support the net flow for another 2 minutes, require twice content of muscle carnitine. Muscle fatigue is a process that involved many components from different points of view: psychological, metabolic and neural (Davis, 1995; Miller, 1995). Some studies showed that in some conditions the exogenous carnitine can decelerate loss of muscle contractile force (Brass et al., 1993). The correlation between metabolic effect of carnitine and increase of muscle fatty acid oxidation, with an increase of ATP synthesis, are remain unclear. So, despite the logic of the case and the availability of some supporting data, the theoretical basis of the efficacy of carnitine for improving muscle function must be explain.

Effect of carnitine on athletic performance

Several studies on the administration of carnitine were different as regards dose and duration of treatment; each of these parameters would provide some different benefit. These studies have involved many peoples who practices different sports and have different experience, age and sex. In addition, they monitoring different parameters as: maximum oxygen consumption (VO_2 max), athletic performance, perceived stress or metabolic parameters (for example

respiratory quotient, lactate accumulation or oxygen consumption at a fixed rate of work). Many studies showed that when supplementation lasted for a short term the effect on athletic performance were negative (Greig et al., 1987 Colombani et al., 1996). Actually, positive effects have been observed just in a long term supplementation. Particularly, it was determined that carnitine decrease the lactate accumulation when combined with exercise (Siliprandi N et al., 1990), again reduced the VO₂ max (Marconi et al., 1985; Vecchiet et al., 1990; Dragan et al., 1987) and also improving the fatty acids oxidation (Natali et al., 1993) (Table 1).

still unknown and requires further investigation to confirm these preliminary results (Huertas R et al., 1992).

Clinical trials on carnitine supplementation

The bioavailability of orally administrated carnitine change roughly from 5 to 15% that is equivalent to small part of the portion that reaches the systemic circulation, in contrast to that assumed up by intravenous (Harper et al., 1988 Segre et al., 1988).

The content of total body carnitine in a 70 kg male is around 128 mmol or 20 g (Brass EP, 1995). A large amounts of it taken orally are required, to change the endogenous reserves. Since the amount of these endogenous

POTENTIAL BENEFITS OF SUPPLEMENTATION OF CARNITINE IN HEALTHY SUBJECTS
<ul style="list-style-type: none"> - Improves fatty acids oxidation; - Decreases the depletion of muscle glycogen; - Change the substrate used by the muscle from fatty acids to glucose; - Activate the pyruvate dehydrogenase; - Increases the production of acetylcarnitine; - Improves resistance to muscle fatigue.

Table 1

The Arenas's study (Arenas et al., 1994) emphasized the different effect of carnitine. It is important to underline that this work studied only athletes engaged in training programs for a period of 1 to 6 months. In these conditions, carnitine associated to training has showed to decrease in muscle carnitine content and simultaneously increased the activity of muscle enzymes, including pyruvate dehydrogenase and the enzymes of the respiratory chain. However, the physiological effect of these changes is

reserves are large, carnitine becomes important parameter in clinical trials. In fact, if 10% of the dose goes in the systemic circulation and 4% of the dose is excreted in the urine, its enough an oral administration of 2 g for day, to induce a net increase of body reserves of 0.12 g per day (Segre et al 1988). So, after two weeks of carnitine administration there is an increase in body reserves of 8%. These estimates, highlight the importance in clinical trials on dosing parameters of carnitine. Cardiac factors or

peripheral muscle may limit the maximum rate of oxygen consumption (VO_2 max) and is dependent on the physiological characteristics of the subjects (Wasserman et al., 1975). During aerobic training, VO_2 max variations occur as a result of alterations in the ability to perform physical tasks.

The assessment of the performance in sports sustained exercise in regimens of resistance or specific activities have been used for groped to provide more generalizable results. A multiplicity of factors including the physiological conditions or the metabolic state of the subject affects on these activities; moreover, are less reproducible tests of maximal exercise. In studies on carnitine supplementation, have been analyzed several factors including muscle mass, enzymatic activity, muscle aches, respiratory quotient, oxygen consumption at a fixed workload.

The data available to determine the effects of carnitine supplementation on physical performance, must have adequate statistical basis in order to detect the expected changes. Most of the studies on the effects of carnitine supplementation in healthy subjects have been recently examined (Table 2). Many of these have shown errors in the scientific design. In

period of 7/14 days and in some case, no more than 28 days (Brass EP, 1995).

Furthermore, most of them has included less than ten subjects, and only in a few cases have been evaluated objective physiological parameters as VO_2 max. Only five publications (Table 2) reported data about the effect of carnitine on this parameter. Two studies have been reported an increase in VO_2 max (Marconi et al., 1985; Vecchiet et al., 1990) while other three studies showed no effect (Greig et al., 1987; Wyss et al., 1990; Hulsmsnn et al., 1992).

Particularly, Vecchiet's study, has documented that a single dose of carnitine one hour before exercise, increase VO_2 max. In contrast, Hultman et al underlined that it is extremely difficult to understand the action of a single oral dose of carnitine.

The majority of studies found no effect of carnitine supplementation on metabolism or performance during exercise, considering certain parameters including the respiratory quotient to a fixed workload, the accumulation of lactate or the estimated cash substrate (Marconi et al., 1985; Oyono-Enguelle et al., 1988; Décombaz et al., 1993; Vukovich, 1994; Wyss et al., 1990). XXXX

STUDY	SUBJECT	DOSE	DURATION	EFFECT ON VO_2 MAX
Marconi et al.	6	4g	2 weeks	Increase
Greig et al.	9	2g	2 weeks	No effect
Dragan et al.	4	3g	2 weeks	No effect
Vecchiet et al.	10	2g	1 hour	Increase
Wyss et al.	7	3g	1 week	No effect

Table 2: Effects of carnitine on VO_2 max in healthy volunteers

particular, the orally administration of carnitine has often been carried out for a

The metabolic effects of carnitine supplementation are unclear, so it is unlikely that oral supplementation for a period up to 4

weeks, may change the physical performance in healthy subjects. The administration of carnitine in order to improve performance in athletes involved in specific activities such as competitive swimming or the marathon, has not demonstrated benefits (Trappe et al., 1994; Columbani et al., 1996).

Again the Arenas's studies (Arenas et al 1991 J, Arenas J et al 1994) showed that oral supplementation protracted for a periods from 1 to 6 months, showed a correlation between exercise and a decrease in muscle carnitine content.

Currently studies does not confirm that the use of carnitine may improve exercise performance in healthy subjects. This assumption cannot be considered definitively negative, due to limitations of the available studies. Future studies based on administration for long periods (more than 6 months) optimized by monitoring important parameters, will demonstrate if there is an improvement in the performance of healthy subjects.

Effect of carnitine supplementation in aerobic training

The regular physical activity and carnitine supplementation, have a role in energy metabolism and can improve resistance capacity. Many studies on the combination between the prolonged ingestion of carnitine and physical training, detect some effects that involved cytosolic fatty acid-binding protein (FABPc) and β -hydroxyacyl-CoA dehydrogenase (β -HAD) enzyme, in skeletal muscle.

The storage of carnitine may improve athletic performance; in fact a large number of studies have turned their concern to demonstrate how can increase fat oxidation and reduce the use of endogenous deposits of carbohydrates in endurance exercise afterwards L-carnitine supplementation (Barnett et al. 1994; Brass and Hoppel 1994; Gorostiaga et al. 1989; Heinonen 1996; Heinonen et al. 1992; Maassen et al. 1995; Marconi 1985; Soop et

al. 1988; Trappe et al. 1994; Vukovich et al. 1994; Wyss et al. 1990).

Most of these studies, measured the oxidation of fatty acids during exercise through indirect calorimetry. The distribution of muscle fibers, can be considered an important indicator of oxidative capability. Muscle biopsies were made before and after training following administration; their influence on the fiber composition, were used to integrated the analysis.

A biochemical marker of the capacity of fatty acids oxidation was the cytosolic fatty acid-binding protein (FABPc) detection as an indicator of fatty acids oxidation in the muscle over which β -hydroxyacyl CoA dehydrogenase (β -HAD) works.

Below is a study of 28 subjects in order to demonstrate the real effect on the performance played by carnitine. It included twenty-eight healthy volunteers who were randomly divided into four groups: a control group (CON, n=7 subjects); a group trained without the use of carnitine called "Exercise training" (ET, n=7 subjects); a group only supplemented with L-carnitine called "carnitine supplementation" (CS, n=7 subjects); a group trained with supplementation of L-carnitine called "carnitine Exercise Training" (CET, n=7 subjects).

The subjects on ET and CET groups, were trained for 40 minutes on a cycle ergometer at 60% of maximum oxygen consumption (VO_2 max), at various times during six weeks. The groups supplemented, assumed 4 g/day of L-carnitine as L-carnitine-tartrate. Muscle biopsies were made on vastus lateralis (Bergström, 1962). The sample were store - 80° C until analysis were made.

To highlight the myofibrillar activity, usually the transverse sections obtained from samples previously taken, were mounted on a coverslip and colored (Brooke and Kaiser 1970). Were monitored muscle fibers of type I, IIa and IIx.

Furthermore were determined through an image analyser, the composition or cross-section, the type of muscle fiber, the area of circulation, the relationship of the capillary fibers and capillary density.

In control group, the activity of β -HAD was 2.20 ± 0.51 mmol g/min. The activity of β -HAD, did not show any change in the ET group either as a result of regular exercise.

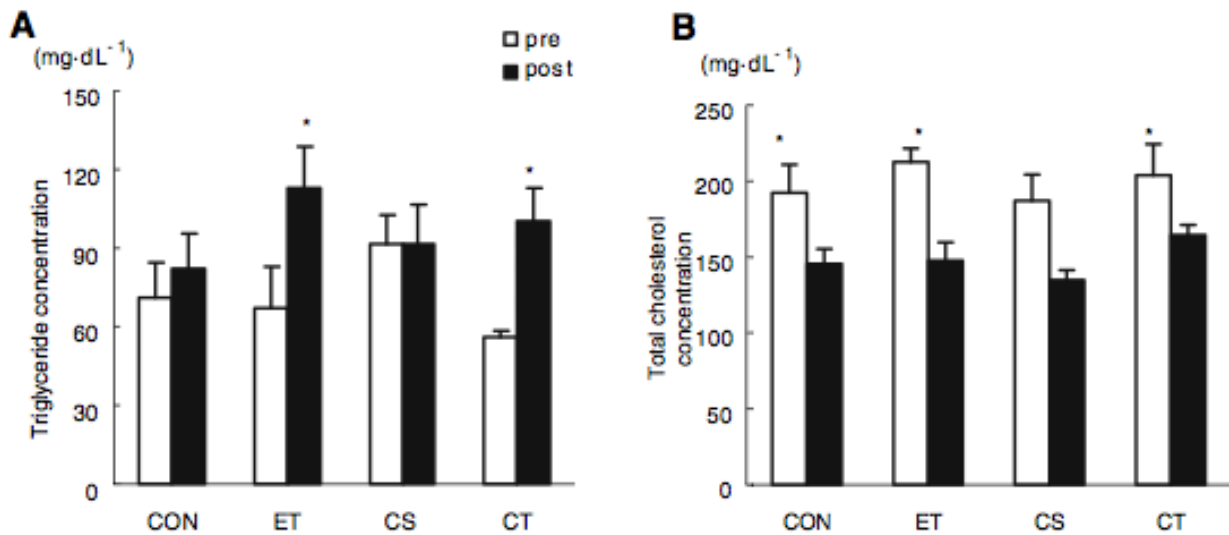


Figure 2: Concentration of total cholesterol (A); Activities of the β -HAD (B)

After treatment, the serum concentrations of triacylglycerols were increased almost twofold for groups ET and CET, while the subjects who took L-carnitine (group CS) showed no significant effect of triacylglycerols serum concentrations (figure 2A). The total serum cholesterol was decreased in ET and CS subjects, but not in CET (figure 2B).

The proportion of type I fibers, after 6 weeks of training, has remained unchanged in all groups except for group ET which showed an increase of 33% versus a decrease of the type II fibers (28%). The distribution of the fibers type IIa, only in the CET group there were an increased of the type IIx fibers; also, it was observed an increase in muscle fibers of the type IIa.

After training the content in FABPc, remained unchanged nevertheless tends to increase in the ET group (35%) and decreases in the subjects of CON group (21%). In all groups the activity of β -HAD was unchanged.

When carnitine has been ingested for 6 weeks were obtained similar results and there was a 18% increase in the activity of β -HAD.

Through this study it was shown that supplementation of carnitine had an additive effect on the phenotypic changes, muscle fiber type and size when combined with the training activity, but did not induce any further positive effect on the metabolism of fats. The concentration of carnitine in the muscles remain unchanged after its supplementation.

It was also demonstrated that, even the serum level of carnitine (after 3 days of the assumption of L-carnitine), the concentration of acyl L-carnitine remain the same confirming the results discussed above (Barnett et al., 1994; Brass et al., 1994; Natali et al., 1993; Trappe et al., 1994; Vukovich et al., 1994; Müller et al., 2002). Despite these positive changes, there was no increase in VO_2 max, which is used to evaluate aerobic

capacity (Colombani et al., 1996; Trappe et al., 1994).

In this study the physical exercises does not reduce the values of serum lipids. The prolonged aerobic exercise reduced serum lipid concentration (Kim et al, 2004; Cha et al., 2003). In subjects of the ET group increased the percentage of type I muscle fibers, while remained unchanged in the CS group. The muscle fibers IIa increased in CET group, although at the expense of the decrease in muscle fiber type IIx.

The distribution of the different types of muscle fibers among the groups CON, CS, and ET were unchanged, except for CET group in which were observed an increase in muscle fiber type IIa and IIx.

This study conforms with the research of Sandra et al. (2002), demonstrated that the administration for three months of L-carnitine (2g for 2 times per day) did not induce any significant change in the composition of the muscle fibers.

Furthermore, suggest that L-carnitine can lead to variations of the characteristics in the fibers fast-twitch when combined with exercise. The main stimulus is the training activity and physical stress, able to induce changes in the composition of the muscle fiber type observed in subjects of the group ET.

During endurance exercise, the increased oxidation of fatty acids in the muscle trained, it can be induced by increased activity and expression of FABPc protein in the cell membrane (Kiens et al., 1993; 1997, Turcotte et al., 1992).

The content of FABPc depends on external conditions such as training and the use of greater quantities of combustible. Higher concentrations are favorable to the characteristics of fast-twitch fibers (Clavel et al., 2002).

The content of FABPc, had a trend of increase (53%) following the training prolonged (even if not statistical significant), while the content of FABPc did not change afterwards carnitine supplementation. This results suggest that

carnitine does not exert a deep effect on the expression of FABPc, which rather is correlated with exercise. To induce an increase in activity of the enzymes involved in both the citric acid cycle and β -oxidation process is necessary to practice regular physical activity (Gollick and Saltin, 1982). However, contradictory results were obtained for example from another study in which a training group was able to increased β -HAD activity (Kiens et al., 1993); but until now, there are nomore other studies that confirm this result (Bylund et al., 1977; Schantz et al., 1983; Wibom et al., 1992; Lee et al., 2001). In summary, a phenotypic effect on the expression of the fibers and in the size of the muscles, can be induced by carnitine supplementation only in muscles that have glycolytic characteristics.

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